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IDENTIFICATION AND CHARACTERIZATION OF FUNGAL PATHOGENS ISOLATED FROM INFECTED VEGETABLES OF MALABAR REGIONS OF KERALA

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Chapter 1: Introduction

Vegetables are essential sources of vitamins, minerals, dietary fibres, oils and fats in human nutrition. They are the fresh and edible portions of herbaceous plants (Slathia *et al.,* 2021). They are important food and highly beneficial for the maintenance of health and prevention of diseases. They are valued mainly for their high carbohydrate, vitamin and mineral contents. There are different kinds of vegetables; they may be edible roots, stems, leaves, fruits or seeds. Each group contributes to diet in its own way (Robinson, 1990).

Vegetables contribute towards food and nutritional security of the people. With the focused attention given to the vegetable sector, there has been spectacular change in terms of production and availability of vegetables. India is now the second largest producer of vegetables after China, contributing about 14.45 per cent of the total world production (Sant *et al.,* 2015). Many vegetables are recognized as sources of natural antioxidants like phenolic acids, tannins, flavonoids and other metabolites (Atawodi, 2005). Secondary metabolites present in plant foods, such as alkaloids, phenolic compounds (flavonoids, isoflavonoids and anthocyanins) and terpenoids, have gained importance because of their antioxidant, antiviral, antibacterial and anticancer effects (Makkar *et al.,* 2007). The importance of fruit and vegetables in human nutrition cannot be overestimated as it provides essential growth factors such as vitamins and minerals necessary for proper body metabolism (Al-Hindi *et al.,* 2011). Humans and many animals have become dependent on fruits and vegetables as a source of food (Lewis, 2002).

Kerala state is the southernmost region in India; geographically, it is smoothed with the affluence resources such as backwaters, rivers and streams, pleasant sunshine and humidity, rainfall, and a right climate suitable for everyone and also a moderate weather throughout the year. The geographical area of Kerala spreads over the Western Ghats which is famous for its flora and fauna diversity and well known as the ‘Biodiversity Paradise'. Also, the palm-fringed Arabian Sea in the west, the majestic Western Ghats with in the east, lush green paddy fields, charming backwaters, year-round colourful festivals all make Kerala a paradise in the world. Such a topographical feature of the state helps the farmers to cultivate some of the important cash crops that are particularly apt for the soil quality of the state (Viswanathan, 2021).

Malabar, region of Kerala, is the wettest and biodiversity-richest region in the country. Agriculture has been practiced in the region from ancient times, involving the majority of the people. The Malabar region, which broadly consists of parts of the coastal plain of Karnataka and most of Kerala, between the Western Ghats range and the Arabian Sea. The landscape of the region is characterized by an asymmetrical topography that includes lowlands bordering the sea, the midlands dominated by undulating subdued hills and steep scarp slopes and forests, and the highlands on the east, in parts of the Western Ghats. The altitude ranges from below mean sea level to 2694 meters above mean sea level. The highest peaks of the Western Ghats, Anamudi (2695 m) and Dodabetta (2636 m) are part of the region (Singh, 2014).

The yield of vegetables is reducing gradually every year due to the soil-borne fungi. It is facing several biotic problems and is under threat due to soil borne pathogens in all over vegetable growing areas. Soil-borne plant diseases cause significant damage to almost all crops particularly to the vegetables (Usman *et al.,* 2013). Fruits and vegetables are suitable hosts for many rot-causing fungal pathogens leading to substantial postharvest losses (Ahmad *et al.,* 2020). During post – harvest handling even in developed countries, it is estimated that about 20 – 25% of the harvested vegetables and fruits are decayed by pathogens (Droby, 2005; Zhu, 2006). In developing countries, up to 35% of postharvest losses are estimated to be attributed to improper storage facilities (Kitinoja *et al.,* 2019).

There has been an increase in the need to identify and isolate the fungi associated with their spoilage of vegetables. Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture. Colonization of fungi is a critical phase in the microbial spoilage of post harvested vegetable. The colonization process involves the ability of the microorganism (fungi) to establish itself within the (host). The contamination of vegetables by fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Jonathan *et al.,* 2010). Now disease deals as interaction of "host pathogen suspects". Thus, the development of a plant disease depends upon three aspects, a susceptible host, a virulent, pathogen and a favorable environment. These aspects in sufficient duration allow infection by pathogen and cause subsequent development of disease in plants (Hemendra and Sushil, 2011).

Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Tournas, 2005). According to Junghare *et al.,* (2014) fungi is the plural of the word fungus which is derived from the Latin word FUNGOUR which means to flourish. The fungi may be defined as non-green, nucleated thallophytes. However, mycologists have defined fungi more scientifically. The fungi include nucleated spore bearing achlorophyllous organisms that generally reproduce and whose filamentous branched somatic structures are typically surrounded by cell walls containing cellulose or chitin or both. A more technical definition of fungi was later given by which says that fungi are chlorophyl less non-vascular plants whose reproductive or vegetative structures do not permit them to be assigned to positions among recognized groups of higher plants or algae. Fungi grow in diverse habitats. Majority of fungi prefer to grow in darkness and dim light in most habitat (Naik *et al.,* 2010). Phosphorus playing a key role in the development and yield of crop plants and it is an important plant nutrient. Through the processes of acidification, chelation and exchange reaction, phosphate solubilizing micro-organisms (phosphate solubilizing bacteria, fungi and actinomycetes) convert these insoluble phosphates into soluble form (Reyes *et al.,* 2002). The diagnosis of plant pathogenic fungi is one of the urgent needs due to its importance in reaching rapid and efficient disease management systems to reduce or prevent damages caused by the fungal infections (Balodi *et al.,* 2017). Molecular biology has offered a number of insights into the detection and enumeration of fungal pathogens and information on identifying unknown species from their DNA sequences. It is an accurate method for the identification of fungal pathogens (Ziedan *et al.,* 2018). It is difficult to identify theses pathogens at the species level (Machouart *et al.,* 2004). Thus, finding efficient, dependable, and reproducible assays to confirm the pathogen species became crucial. Accordingly, PCR-based techniques provide excellent results in a shorter time (less than a week) than the conventional techniques (several weeks or months), allowing for an early preventive reaction to the infections' risks (Abdulrahman and Haleem, 2023).

Plant-pathogenic fungi produce an array of extracellular hydrolytic enzymes that enable them to penetrate and infect the host tissue; these enzymes are collectively called cell wall degrading enzymes (CWDE). They may contribute to pathogenesis by degrading wax, cuticle and cell walls, thus aiding tissue invasion and pathogen dissemination (Kikot *et al.,* 2009). The cell wall degrading enzymes (CWDEs) are one of the main pathogenic factors of plant pathogenic fungi, mainly including pectinases and cellulases (Perincherry *et al.,* 2021). After infecting plants, pathogens can secrete hydrolytic enzymes such as Cx, βG, PMG, PG, PGTE, and PMTE to destroy the host cell

wall and inhibit other defense systems, including the host resistance reaction (Ezzat *et al.,* 2021). Plant cell wall polymers serve as a potential barrier to the penetration and spread of pathogens and as substrates for extracellular enzymes secreted by pathogens (Annis and Goodwin, 1997). Due to its safety and biodegradability, and environmental compatibility, chitosan has been found to have many applications in biology, cosmetics, food, wastewater treatment, heavy metal recovery, photographic chemistry, textile weave, printing, dyeing, and paper making (Muzzarelli, 1977; Hon, 1996). Chitosan has antibacterial activity and has been used in food and fruit preservation (No *et al.,* 2002). Fungicidal potential of chitosan has been reported against many pre- and postharvest diseases of horticultural commodities (Bhattacharya, 2013). This is possibly because of the elicitor properties of chitosan (Benhamao and Theriault, 1992). Fungicidal value of chitosan has been proven both at *in vitro* and *in vivo* applications (Bautista-Banos *et al.,* 2006). Elicitors like chitosan can be used as an alternative method of control of fungal diseases because elicitor treatments cause reduced environmental hazards compared to conventional pesticides (Holopainen *et al.,* 2009).

Medicinal plants are rich sources of antimicrobial agents. Many infectious diseases have been known to be treated with herbal extracts. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Although many plant species have been tested for antimicrobial properties, the majority of them have not been sufficiently evaluated (Mishra *et al.,* 1985). The development of microbial resistance towards antibiotics has heightened the importance of the search for new potential effective plants and plant constituents against pathogenic microorganisms (Ahmad *et al*., 2006). The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others (Nonita *et al*., 2010). Medicinal plants are used to treat different microbial infections. The World Health Organization reports that various plant fractions and their dynamic constituents are used as traditional medicines by 80% of the world population (Shahat *et al.,* 2018). Medicinal plants are used by many ethnic groups as a source of medicine for the treatment of various ailments in both humans and domestic animals. These plants produce secondary metabolites that have antimicrobial properties; thus, screening of medicinal plants provide another alternative for producing chemical fungicides that are relatively non-toxic and cost-effective (Salome *et al.,* 2016). There are few different classes of effective antifungal drugs available for the treatment of

fungal diseases of plants, animals and humans. Thus, it is important to develop new sources of antifungal agents. Further development of antifungal compounds with diverse chemical structures and novel mechanisms of action is necessary because there has been an alarming increase in the incidence of new and re-emerging infectious diseases as well as resistance to currently used drugs (Chang *et al.*, 2006). Antimicrobial resistance to commercially available medications has become a worldwide problem in recent years. Similarly, several species are developing resistance to currently available antifungal medications. This shows that there is a need to investigate other options. In terms of confirming traditional usage and assessing phytochemical content, medicinal plants have become the focus of significant research (Gizaw *et al.,* 2022).

Review of Literature

Vegetables have been a topic of interest in India and abroad as because of their nutritive value hardiness and medicinal significance. Reports of pathogens and their pathogenesis on the vegetable crops have also been researched all over the world (Wanjiku *et al.,* 2020). A fungus is one of the most diverse microorganisms that inhabit different environmental sources such as soil, plant parts (leaves, root and fruits), water and food sources (Sartori *et al.,* 2013; Rebecca *et al.,* 2012). The growth and distribution of fungi are affected by different environmental factors such as temperature, pH, moisture, degree of aeration, amount and type of nutrients (Gaddeyya *et al.,* 2012). The distinguishing characteristics of this group as a whole are that, they are eukaryotic, non-photosynthesis, lack tissue differentiation, have cell wall of chitin and glucan or other polysaccharides and propagate by spores (sexual and /or Asexual spores) (Hwang and Benson, 2002). The real number of fungi is still unknown; on the other hand, only 5-13 % of the overall evaluated worldwide fungal species have been characterized (Maheswari and Komalavalli, 2013). Thus, the fungal Isolation and identification from the different environmental sources is still very essential for the viewing and recognizing of more species, editing scientific classification, evaluating their effects in nature, and supplying strains for ecological remediation, biological control, and industrial aspects (Blackwell, 2011).

Fungi play a substantial role in the spoilage of vegetables, because of their pathogenicity to the harvested products. However, during the various stages of pathogenesis, some of these fungi may generate different mycotoxins, toxic to humans and animals that consume them. During recent decades a variety of vegetables that form part of our daily diet have been added to the list of products exposed to mycotoxin contamination. Since *Aspergillus, Penicillium* and *Alternaria* sp., are the

major mycotoxigenic fungi that attack harvested vegetables, a variety of mycotoxins produced by these fungal genera during pathogens is may contaminate the vegetable tissues (Barkai and Paster, 2008).

It has estimated that between 10 and 30% of severe economic crop yield reduction was due to root, stem, and ear rot disease caused by *Fusarium* species (Uhlig *et al.,* 2007). Fungal diseases harm the fruit even in field and reduce the yield. But due to characteristics of latent infection, huge losses of commercial value results after harvest (Sossah *et al.,* 2019). Of the world's major crops, plant diseases result in an annual estimated loss of 10–15% with direct economic losses of up to hundreds of billions of dollars (Chatterjee *et al.,* 2016). About 70–80% of diseases are caused by plant pathogenic fungi having adverse effects on growth and yield of crop (Li *et al.,* 2017). In recent years, crop yield and quality have been severely affected by fungal diseases as they have become increasingly serious and they have become an important bottleneck for the sustainable agricultural development (Marin-Menguiano *et al.,* 2019).

Phytopathogenic fungi cause huge losses in agricultural production as they decrease crop yield and quality. Farmers must use many synthetic chemical pesticides to prevent the occurrence of crop diseases and insect pests. A series of environmental and ecological problems such as the increase in resistant weed populations, soil compaction, and water pollution (which seriously affect the sustainable development of agriculture) have been resulted due to the extensive use of these pesticides (Peng *et al.,*2021). In many instances, both quality and productivity in various commercial crops including vegetables are declined by these plant pathogenic fungi (Iqbal *et al.,* 2018). In terms of quality and productivity, vegetables suffer huge losses from several fungal and bacterial diseases. Around 85% of the diseases of vegetables are caused by fungi or fungal-like pathogens. Losses are more severe in developing than developed nations of the world (Enyiukwu *et al.,* 2014).

A total of six different fungi such as *Alternaria alternata, Aspergillus niger, Alternaria solani, Geotrichum candidum, Fusarium oxysporum* and *Rhizopus stolonifer* were found to be associated with post harvest tomato. Higher infection was recorded for *A. solani* followed by *A. niger* and *G. candidum*. The lowest infection percentage was observed for *F. oxysporum* followed by *A. alternata* and *R. stolonifera*. *A. solani* produced aerial mycelium with yellowish to reddish diffusible pigments. *A. niger* cultures were typically black and colonies were initially whitish to yellow and later became brown to black in colour. *G. candidum* produced white and nonaerial colonies. *F.*

oxysporum produced circular, aerial mycelium initially white, later changed to light pink. *R. stolonifer* produced whitish to grey fuzzy colonies (Nizamani *et al.,* 2021).

Fatima *et al.,* (2009) reported that *Alternaria alternata, A. citri, Aspergillus niger, A. flavus, Aspergillus* sp., *Cladosporium cladosporioides, Drechslera australeinsis, Fusarium solani, Fusarium* sp., *Geotrichum candidum, Penicillium* sp., *Phytophthora capsici* and *Rhizopus stolonifer* were responsible for postharvest deterioration of vegetables. Different fungi such as *Alternaria alternata, Alternaria, Rhizopus, Mucor, Aspergillus niger, Aspergillus flavus, Cladosporium, Botrytis cinerea, Fusarium moniliforme, Drechslera, Penicillium,* and *Geotrichum candidum* were identified among various vegetables including potato, carrot, okra, eggplant, turnip, cucumber, round gourd, cauliflower, and chilli (Parveen, 2021). Twenty two different species representing seven different genera of fungi were isolated from different vegetables such as tomato, potato, cucumbers, eggplant, pepper, onion and Squash; the most frequently isolated fungi were *Rhizopus* sp., followed by *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp. and *Alternaria* sp. while the least frequently isolated fungal genera were *Rhizoctonia* sp. in addition to yeasts (Gafur and Muhamad, 2017). Ten different species such as *Aspergillus flavus, Aspergillus niger, Curvularia, Fusarium* sp., *Mucor* sp., *Mucor mucedo* sp., *Pencillum* sp., *Rhizopus* sp., *Rhizomucor* sp., and *Syncephalastrum racemosum* were obtained (Kavya *et al.*,2020). Iniekong *et al.,* (2015) studied spoiled fruits and vegetables and isolated *Rhizopus stolonifer, Aspergillus niger* and several other species of *Fusarium* which plays a major role in spoilage of consumable fruits and vegetables.

Fungi species of *Aspergillus candidus, A. niger, Aspergillus parasiticus, Aspergillus rugulosus, Aspergillus terreus, Penicillium, Pseudeurotium, Trichoderma* sp. and some mycorrhizal fungi commonly reported to effectively solubilizing phosphorus (Aseri *et al.,* 2009; Muraleedharan *et al.,* 2010). Phosphate solubilizing fungi *Aspergillus* sp. (55.69%), *Penicillium* sp., and *Fusarium* sp. were isolated from haricot bean, fava bean, cabbage, tomato, and sugarcane of Jimma Ethiopia (Firew *et al.,* 2016). The genus *Fusarium* has large and still untapped genetic potential to produce a vast array of novel secondary metabolites. It is a treasure trove of secondary metabolites with unique chemical diversity. Remarkably, most of the chemical diversity and secondary metabolites of *Fusarium* is associated with plant endophytic strains and production of secondary metabolites in fungi and their plant tissues seems substantial orthogonal. Secondary metabolites derived from *Fusarium* have attracted global attention because of their remarkable biological properties and potential application in clinic and in agriculture (Li *et al.*,2020). Fungi

proliferate and may produce secondary metabolites under favorable environmental conditions as and when temperature and moisture are suitable (Brase *et al.*,2009). A broad range from simple to very complex organic compounds are produced by fungal metabolism and most of them exhibit certain biological activities (Panaccione, 2005).

The fungal isolates such as *Rhizoctonia solani* and *Fusarium solani* were identified from seedling damping-off disease of *Cucumis sativus* (Ziedan *et al.,* 2018). A total of eight fungal species viz., *Aspergillus niger, Aspergillus tubingensis, Alternaria tenuissima, Alternaria alternate, Alternaria gaisen, Rhizopus stolonifer, Penicillium citrinum,* and *Fusarium oxysporum* were identified from different vegetables by ITS sequencing (Bukhari *et al.,* 2018). Modern microbial phylogenetic reconstruction methods must, ideally, be extremely versatile: individual sequencing projects can span thousands of closely related isolate genomes, metagenomes can produce hundreds of high-quality but quite diverse metagenome-assembled genomes (MAGs), and cheap sequencing regularly yields thousands of new genomes (Pasolli *et al.,* 2019). Jointly accommodating closely related and deep-branching microbial genomes is thus difficult, especially at large scale. In little more than a decade, pan-microbial phylogenies have grown from 191 genomes 56 to more than 10,000 species (Zhu *et al.,* 2019).

Industrially relevant enzymes have enhanced interest in the exploration of extracellular enzymatic activity in several microorganisms as biotechnological sources (Abu, 2005). Enzyme cellulase provide a huge opportunity in the field of biomass utilization (wen, 2005). To get optimum enzyme activity, temperature must be controlled as another critical parameter because temperature is a significant controlling factor for enzyme production **(**Akintobi *et al.,* 2012**)**. At certain temperature, enzyme activity is usually optimized and highly stabilized and above or below temperature, the enzyme becomes in active, slow or entirely denatured (Lehninger *et al.,* 1992). Optimum temperature of pectinase activity by *Aspergillus niger* and *Aspergillus flavus* was 40°C. Different species of *Aspergillus* have shown variations in optimum temperature (30- 400 °C) for pectinase activity, and it was regarded as the best range (Khatri *et al.,* 2015).

In general, the presence of enzymatic activities of these fungal isolates indicated the importance of cell wall–degrading enzymes in pathogenesis. This is in congruence with De Bary (1886) who postulated the involvement of cell wall–degrading enzymes in plant disease more than 100 years ago (Dean and Timberlake 1989). *Macrophomina phaseolina* showed amylolytic, cellulolytic, and xylanolytic activities. *Trichoderma harzianum, Aspergillus niger,* and *Alternaria*

species were reported as amylase, cellulase, and xylanase producers (Kaur *et al.,* 2012). The number of species of *Aspergillus* possess xylanolytic potentials. Most members of the *Aspergillus niger* group are notable producers of extracellular enzymes including important plant cell wall hydrolyzing enzymes such as xylanases (Polizeli *et al.,* 2005). Physical conditions such as temperature and pH of the extracellular environment greatly affects the growth and enzymatic activities of the microorganisms (Hyde *et al.,* 2019). Occurrence of these fungal species is largely dependent on the local environmental conditions as well. Many fungal species, including those present in the air, may be responsible for the production of metabolites with the most varied industrial and biological applications, enzymatic and antimicrobial activities (Viriato, 2014).

Chitosan is a polysaccharide derived from chitin, which is the second most abundant polysaccharide in the world, after cellulose. Chitin is a polysaccharide of animal origin and is the main constituent of the outer skeleton of insects and crustaceans like shrimp, crabs, and lobster. Chitosan is considered one of the most promising products for the control of several important postharvest fungi in fruits and vegetables (No *et al.,* 2005). The application of chitosan and chitin in agriculture is becoming a major focus of plant protection research. Since most chemical fungicides are potentially highly toxic to humans and animals and not very biodegradable, they often cause water and soil pollution. It is reported that the antimicrobial activity of chitosan also depends of the molecular weight, is better when chitosan of low molecular weight and oligochitosan instead of high molecular weight chitosan is applied. In this sense, high molecular weight chitosan cannot pass through the microbial membrane and acts against microbial development (Anirban, 2013). Concerning to the efficacy of chitosan at different concentrations is reported that at lower concentrations, chitosan binds cell surface of microorganisms (negatively charged), disturbing the cell membrane, and causes death of microbial cell by leakage of the intracellular components; however, at higher concentrations, chitosan may coat the microbial surface and prevent the leakage of intracellular components (Seyis and Aksoz, 2005).

Medicinal plants are rich sources of antimicrobial agents. Many infectious diseases have been known to be treated with herbal extracts. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Although many plant species have been tested for antimicrobial properties, the majority of them have not been sufficiently evaluated (Abdallah, 2011). The development of microbial resistance towards antibiotics has heightened the importance of the search for new potential effective plants and plant constituents

against pathogenic microorganisms (Ahmad *et al*., 2006). The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others (Nonita *et al*., 2010).

Over the past decade there has been an explosion of interest in the antimicrobial, particularly antibacterial and antifungal, activity of natural products (Clark and Hufford, 1993). The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases (Cooper *et al*., 2006). Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery (Cseke *et al*., 2006). Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (phytochemicals) (Mongole *et al*., 2010). The effect of plant extracts on microorganisms has been studied by a very large number of researches in different parts of the world (Ohiri and Uzodinma, 2000; Amanlou *et al.*, 2004; Mahesh and Satish, 2008). Medicinal plants are used from prehistoric times. Different extracts obtained from *Piper longum* stem displayed mild activity against most of the fungi. Among the extracts, ethyl acetate extract was found to be active against all the fungal strains and was strongly active against *Fusarium* sp. The fungus such as *Penecillum* sp. was resistant against all the crude extracts and all the crude extracts showed higher antifungal effect against *Mucor* sp. (Ali *et al.*, 2007).

Significant antifungal activity of ethyl acetate and aqueous extracts of *S. rhombifolia* against *Aspergillus niger, Candida albicans* and *Microsporium gypseum* has been recorded (Singh and Singh 2018). The methanol extract of the entire plant of *S. rhombifolia* was reported to exhibit antifungal activity against *Candida albicans* and *Cladosporium cucumerinum* and also exhibited antifungal activity against *Aspergillus niger, Microsporium gypseum*. Leaf extracts of selected South African plant species viz., *Bucida buceras, Breonadia salicina, Harpephyllum caffrum, Olinia ventosa, Vangueria infausta* and *Xylotheca kraussiana* were investigated for antifungal activity against selected phytopathogenic fungi. *Bucida buceras* showed the best antifungal activity against selected fungi (Mahlo, 2016). Some medicinal plant extracts of methanol and ethyl acetate were screened for their antifungal activity against nine different fungi. Among these ethyl acetate

extracts of *Adhatoda vasica, Ocimum sanctum* and *Holoptelea integrifolia* exhibited maximum antifungal activity against *Alternaria* sp., *Aspergillus parasi, Aspergillus nidulans, Trichoderma harzianum* and *Aspergillus flavus*. Ethyl acetate extracts showed promising antifungal activity against *Adhatoda vasica, Ocimum sanctum* and *Holoptelea integrifolia* against *Aspergillus nidulans*, and *Alternaria* sp. might be applicable as fungicide against fungal plants disease (Ishnava *et al.*, 2012). *Aristolochia indica* inhibited the growth of 11 microorganisms such as *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Bordetella bronchiseptica, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus faecalis, Candida albicans, Aspergillus niger* and *Saccharomyces cerevisiae* (Kumar *et al.*, 2006). Different solvent extracts (Methanol, chloroform and aqueous) of *Cissampelos pareira* were evaluated for their antibacterial activity against selected clinical pathogens. Methanol extract of *C. pareira* showed significant antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumonia* (Seetharaman *et al.*, 2018).

Statistical analysis

The experimental results were expressed as mean ± Standard Error Means (SEM) of triplicates. Analysis of data was carried out by applying one-way analysis of variance (ANOVA) using SPSS. P-value lesser than 0.05 ($P< 0.05$) was considered as statistically significant.

Scope of the study

Vegetable intake can promote health and prevent chronic diseases such as heart diseases and certain types of cancer. The health benefits of fruits and vegetables seen in epidemiological studies are the main reasons for the recommended intake of at least 400 g of fruit and vegetables per day. Vegetables have been a topic of interest in India and abroad as because of their nutritive value hardiness and medicinal significance. Pathogens and their pathogenesis on the vegetable crops have also been researched all over the world. The indigenous people have helped in conservation of biodiversity. Ethnic people need to be survived for identification of plants associated with various ethno-botanical uses followed by phytochemical studies. Though the extensive research has been done in the area of fungi causing diseases to vegetables and other agricultural crops is it very essential to study in detail. Development of molecular diagnostic tools for early detection and development, characterization, studying and managing diseases caused by Fungi. Identification and development of biocontrol formulations is also very much needed for the effective management of fungal diseases and the formulations should be properly evaluated in farmers field and demonstrated

to the farmers. Integrated management using cultural methods, bio agents, plant products and resistant cultivars should be adopted. The fungal pathogen being a soil borne pathogen can be managed effectively by cultural methods like crop rotation which should be encouraged among the farmers. Farmers also should be trained on identification and management of various diseases caused by fungal species by experts, scientists and extension functionaries of state and central government organizations.

Objectives

- Isolation, identification and morphological characterization of fungi isolated from infected vegetables and soil samples.
- Molecular characterization of the isolated fungi.
- Identification of secondary metabolites from fungal isolates
- Evaluation of cell wall degrading enzyme activity of fungal isolates
- Antifungal properties of botanicals and bioassay of chitosan against isolated fungi.

Chapter 2: Isolation, identification and morphological characterization of fungi from infected vegetables and soil samples.

Materials and Methods

2.1 Sample collection and processing

Healthy and infected vegetables *viz., Cucumis sativus, Coccinia grandis, Momordica charantia* and *Trichosanthes cucumerina* were collected from the agriculture field of Malabar regions of Kerala, India during the month of September 2020 to November 2020. The samples were collected in a separate sterile polythene bag and brought to the laboratory for the further studies. Infected samples surface was washed with tap water followed by distilled water to remove dirt particles. Soil samples were collected from the root region of vegetable field by digging 10-15 cm depth under the soil. The soil was dark brown sandy loam; moderate. The collected samples were put into sterile polythene bags and stored at 4℃ until further analysis.

Cucumber (*Cucumis sativus* L.) plants are cultivated throughout the world. The fruit, which are formed from an inferior ovary. Flesh tissue of the fruit at this stage is crisp, and seeds remain small and tender, allowing for consumption of the full fruit. Unlike fruits that are eaten ripe, where metabolic attributes such as sweetness, flavor, and aroma are key quality determinants, the predominant characteristics that drive cucumber fruit quality are morphological: size and shape;

external features, such as waxiness, spines and warts; and internal features, such as flesh thickness and seed cavity size (Grumet *et al.*,2022).

Bitter gourd (*Momordica charantia*) is an important vegetable crop and is grown for its immature tuberculate fruits which have a unique bitter taste. The fruit is a pendulous broadly ovoid and beaked berry. Immature fruits are green in colour, and then become reddish-orange when ripe. They split open at maturity to release the seeds sheathed in a sticky red pulp (Goo *et al.*,2016).

Snake gourd (*Trichosanthes cucumerina*), cultivated for its oddly shaped edible fruits. The narrow fruits often reach 1.5 metres (5 feet) in length; they are green with white stripes when young, becoming a red-orange colour when mature(Khan *et al.*,2016).

Coccinia grandis (Ivy gourd) is occasionally cultivated as a garden vegetable. The ivy gourd fruit belongs to the berry type: oval and hairless with thick and sticky skin. The raw fruit is green in color and turns bright red when it is ripe. The mature fruit is usually from 25 to 60 mm long by 15-35 mm in diameter and contains several pale, flattened seeds (Nagare *et al.,* 2015).

2.2 Isolation of fungi from infected vegetables and soil samples

A small portion of the spoilt area of the infected vegetables were cut in and out using a sterile scalpel and inoculated onto a freshly prepared PDA plate. Soil dilution technique (Saksena and Mehrotra, 1952) was followed to isolate fungi from soil samples. The inoculated plates were incubated at 30°C for 5 -7 days and were observed for fungal growth and later sub-cultured for another 7 days at room temperature on PDA. Resulting colonies were then sub-cultured onto Potato dextrose agar (PDA) to obtain pure isolates. CFU/mL was also calculated using the formula:

$$\frac{\text{Number of colonies x Dilution Factor}}{\text{Volume of culture plate}}$$

2.3 Morphological characterization of fungi isolated from infected vegetables and soil samples

The pure fungal isolates of both infected vegetables and soil samples were identified based on both cultural and morphological characteristics by using Lactophenol cotton blue staining. Identified fungal species were compared with confirmed representatives of different species (Udoh *et al.,* 2015).

2.4 Pathogenicity test on fresh healthy vegetable samples

Healthy vegetables collected from the same field were washed with clean tap water, followed by distilled water to remove any soil debris. Vegetables were surface-sterilized by dipping in 75% ethanol for about three minutes, rinsed with distilled water, and then air-dried. A sterile cork borer

(5 mm diameter) was used to wound each vegetable and mycelial discs of equivalent diameter obtained from the edge of actively growing pure cultures were placed on the wound (Baiyewu *et al.,* 2007; Chukwuka *et al.,* 2010).

2.5 *In vitro* screening of Tri calcium phosphate (TCP) solubilizing ability of isolated fungi

In vitro screening of tri calcium phosphate (TCP) solubilizing ability was done according to Pikovskaya, 1948. Spot inoculation of fungi in culture plate having Pikovskaya media. A clear zone around growing colony indicates phosphate solubilizing activity.

Results and Discussion

PDA is considered as one of the most common and expensive fungal growth medium and it relies on a potato crop which leads to decreased potato in markets (Oluwanisola and Adebayo, 2009). Several workers stated that PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi (Xu *et al.,* 1984; Maheshwari *et al.,* 1999; Saha *et al.,* 2008). PDA can be too rich in nutrients, most fungi thrive on it and thus encouraging the mycelial growth with ultimate loss of sporulation (UKNCC, 1998; Somappa, 2013; Koley and Mahapatra, 2015).

Fungal species were identified based on the morphological feature such as surface texture (glabrous, suede-like, powdery, granular, fluffy, downy, cottony), surface topography (flat, raised, heaped, folded, domed, radial grooved), surface pigmentation (white, cream, yellow, brown, pink, grey, black etc.), reverse pigmentation (none, yellow, brown, red, black, etc.) and by microscopic observation. A total of 8 morphologically different pathogenic fungi viz., *Pichia kudriavzeii, Geotrichum candidum* (*Cucumis sativus*), *Fusarium globosum, Aspergillus fumigatus* (*Coccinia grandis*), *Ganoderma lucidum, Aspergillus niger* (*Momordica charantia*) and *Aspergillus flavus, Mucor irragularis* (*Trichosanthes cucumerina*) were isolated from four different vegetables. A total of 12 different fungi were isolated from the vegetable field soil. The *Aspergillus* sp. was common in both *Momordica charantia* vegetable and soil sample. It indicates that *Aspergillus* sp. had a role in causing infection in this vegetable. In our investigation the most dominant fungi are *Aspergillus* species and *Cladosporium* species was found in all the four vegetable field soil. Totally, 8 (1×10^{-1}) and 21 (1×10^{-1}) colonies were observed in *Cucumis sativus* followed by *Coccinia grandis* [8 (1×10^{1}), 34 (1×10^{-1}) and 10 (1×10^{-2})], *Momordica charantia* [25 (1×10^{-1}), 24 (1×10^{-1}) and 13 (1×10^{-1})] and *Trichosanthes cucumerina* 13 (1×10^{-1}).

Aspergillus sp. was isolated from *Mangifera indica, Ananas comosus, Musa paradisiaca, Daucus carota* and *Brassica capitata*; *Rhizopus* sp. were isolated from *Musa paradisiaca* and *Brassica capitata*; *Alternaria* sp. and *Cladosporium* sp. were isolated from *Mangifera indica*; *Phytophthora* sp. and *Candida* sp. were isolated from *Ananas comosus* while *Fusarium* sp. and *Mucor* sp. was isolated from *Solanum lycopersicum* (Choudhury and Saikia, 2022). Due to the changes in climatic condition, fungal attack, inadequate rainfall, pests etc. fruits and vegetables are spoilt in recent years and it has become essential to isolate and identify the fungal species associated with spoilage of fruits and vegetables. *Fusarium* sp., *Mucor* sp., and *Rhizopus* sp, were responsible for causing soft rot of tomato and black depression of carrot was from *Aspergillus* sp. (Agrios, 2005). Through direct contact with soil, dust, water and by handling at harvest or during postharvest processing, fruits and vegetables are exposed to contamination by microorganisms. This makes them harbor a wide range of microorganisms including plant and human pathogens (Angela *et al.*, 2010). *Alternaria solani, Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, A. terrus, Aeromonium fusidiocles, Cladosporium* sp., *Drechselra hawaiiensis, Eurotium berbanbrum, Fusarium oxysporum, Macrophomina phaseolina, Penicillium commune, Rhizoctonia solani, Trichoderma harzianum,* and *Ulocladium* sp., etc. are the most frequent associated fungi isolated from the soil of vegetables. These fungi were most prevalent in the soil of fields and also found to be responsible for most of the decline of the vegetable crops (Usman *et al.,* 2014). Presence of *Aspergillus flavus* (48.7%), *Aspergillus nidulans* (20.5%), *Aspergillus sydowii* (17.9%), *Chaetomium globosum* (7.7%), *Curvularia americana* (2.6%), and *Fomitopsis meliae* (2.6%) were observed from the fungal contamination of dried tomatoes. Since *Aspergillus flavus* is known for the production of aflatoxins, the diversity of these fungi is of great public health concern (Kinyungu *et al.,* 2019). *A. flavus* in dried tomatoes, it has also been reported in other Fruits such as cucumber, watermelon, and chili also have the occurrence of *A. flavus* (Jimeta *et al.,*2022).

All fungi inoculated in the healthy vegetables started showing the diseased symptoms from day 0 to day 7. *Ganoderma lucidum, Aspergillus niger Fusarium globosum, Aspergillus fumigatus, Pichia kudriavzeii,* species were able to produce more pathogenic characteristic features on healthy samples from day 1 to day 7. *Mucor irragularis, Aspergillus flavus* and *Geotrichum candidum* species showed less pathogenicity. Disease development was observed every day, and lesion size was measured on the 7th day. After 7 days of inoculation the following symptoms appeared on the healthy vegetables.

Pichia kudriavzeii- Fruits become watery, soft with growth of mycelia.

Geotrichum candidum- Whitish mycelia growth

Fusarium globosum- Yellowish softy appearance with white mycelia growth on the anterior end.

Aspergillus fumigatus-Fruits become reddish with growth of black mycelia.

Ganoderma lucidum-Fruits become spongy yellowish and softy appearance.

Aspergillus niger-Fruits become spongy with growth of mycelia, watery with gas production.

Aspergillus flavus- Fruits become soft with growth of mycelia on the tip.

Mucor irragularis- Whitish mycelia growth with a cream white color.

No symptoms were observed on the control vegetables. Tissues from the infected samples were isolated on PDA plates and morphologically identified. Koch's postulates were fulfilled if the fungal isolates from infected samples were morphologically similar to the original inoculated isolates.

The highest level of deterioration was observed in *Fusarium* which was the most pathogenic fungus and *Aspergillus* species were found to be weakly pathogenic to cucumber fruits (Akinmusire, 2011). Postharvest deterioration of fresh fruits and vegetables are caused by *Alternaria alternata, A. citri, Aspergillus niger, A. flavus, Aspergillus* sp., *Cladosporium cladosporioides, Drechslera australeinsis, Fusarium solani, Fusarium* sp., *Geotrichum candidum, Penicillium* sp., *Phytophthora capsici* and *Rhizopus stolonifer* (Fatima *et al.,* 2009). Studying pathogenic toxins and their mechanisms of pathogenicity is of great significance for understanding the interaction between plant hosts and pathogens, as well as for the use of pathogenic toxins to identify plant disease resistance, to screen for disease resistant mutants, and to control disease (Adam *et al.,* 2018). During the infection process of pathogenic fungi, natural factors such as wind, rain, and the frequency of contact between insects and fungi are important factors that affect the pathogenesis of the host (Gomez *et al.,* 2018). Fungal species viz., *Alternaria alternata, A. solani, Aspergillus flavus, A. niger, Fusarium oxysporum, Pencillium* sp., and *Rhizopus stolonifera* were isolated (from tomato fruit rot), *A. alternata, A. tenuissima, A. flavus, A. niger, Colletotrichum capisi, Penicillium sp.* and *R. stolonifera* (from chilli fruit rot) and *A. alternata, A. flavus, A. niger, F. solani, Penicillium sp.*, and *R. stolonifer* (from eggplant leaf spots) have been reported (Pahnwar *et al.,* 2023).

All isolated fungal pathogens showed *in vitro* tricalcium phosphate solubilizing ability. Phosphorus is one of the major bio elements limiting agricultural production. In agricultural soil,

about 95 to 99% phosphorus is in unavailable form for plant growth. Phosphate solubilizing microorganisms can increase the phosphate availability in soil (Gizaw *et al.,* 2017). For solubilization of inorganic phosphorus, filamentous fungi particularly *Aspergillus niger* and some species of *Penicillium* from non-polar habitats have been tested (Pandey *et al.,* 2008).

Chapter 3: Molecular characterization of isolated fungi

Materials and Methods

3.1 DNA extraction, sequencing, and phylogenetic analysis

DNA was extracted from the mycelial mats of individual fungal isolates using the DNeasy Plant Mini Kit (Qiagen, USA). To amplify the complete sequence of 5.8 S ribosomal RNA gene, and- I and II spacer regions completely and partial sequence of 18S and 28S rRNA gene regions of the pathogen, the ITS6 (5'GAAGGTGAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primer pairs were used (Cooke *et al.,* 2000; White *et al.,* 1990). PCR, gel electrophoresis, elution of bands, sequencing and sequence analysis was conducted (Prathibha *et al.,* 2018). Using the neighbour-joining (NJ) method, a phylogenetic tree was constructed based on ITS sequence of isolate with the software MEGA -X version 10.2.0 (Saitou and Nei, 1987; Kumar *et al.,* 2018).

Results and Discussion

ITS gene sequencing results confirmed the genus and species of fungal isolates. Similar sequences were retrieved from the GenBank by following BLASTN analysis and a phylogenetic tree was generated using the neighbour-joining method. The phylogenetic tree analysis confirmed to show 90 percent similarities with other retrieved fungi.

The diagnosis of plant pathogenic fungi is one of the urgent needs due to its importance in reaching rapid and efficient disease management systems to reduce or prevent damages caused by the fungal infections (Balodi *et al.,* 2017). Molecular biology has offered a number of insights into the detection and enumeration of fungal pathogens and information on identifying unknown species from their DNA sequences. It is an accurate method for the identification of fungal pathogens (Ziedan *et al.,* 2018). Different fungal species were isolated from soil and plant parts and the PCR products were sequenced, compared with the other related sequences in GenBank (NCBI). *Aspergillus niger, Aspergillus tubingensis, Alternaria tenuissima, Alternaria alternate, Alternaria gaisen, Rhizopus stolonifer, Penicillium citrinum,* and *Fusarium oxysporum* were identified from soil and plant parts (fruits and leaves). Using PCR products identified species were sequenced and

compared with other related sequenced species in GenBank (NCBI) (Alsohaili and Bani-Hasan, 2018). For providing phylogenetic information, Internal transcribed spacers (ITS) have been verified as the best marker genes for all fungus classes, and as the recommended technique (Nilsson *et al.,* 2009). With the same primers ITS1 and ITS4, ten isolates of *Trichoderma* spp., were analysed for obtaining amplified DNA fragment sizes of 600 bp (Garcia-Nunez *et al.,* 2017). Using primers ITS1 and ITS4, a single PCR product of approximately 560–600 bp from 17 biocontrol isolates of *Trichoderma* spp. was obtained (Hermosa *et al.,* 2000).

Chapter 4: Identification of secondary metabolites produced by *Fusarium globosum*

Materials and Methods

4.1 Preliminary qualitative analysis of fungal metabolite

Methanol and ethyl acetate extracts of *F. globosum* was used to isolate secondary metabolites by following method (Swathi *et al.,* 2013).

4.2 Quantitative analysis of fungal metabolite

Total phenolic content was estimated according to Folin-Ciocalteu's phenol reagent (Adedapo *et al.,* 2009; Nabavi *et al.,* 2008), Total flavonoid was estimated Colorimetric aluminum chloride method (Ebrahimzadeh *et al.,* 2001; Nabavi *et al.,* 2008) and Tannin content was estimated by the method of Price and Butler (1977).

4.3 *In vitro* Antioxidant activities of fungal metabolites isolated from *F. globosum*

Total antioxidant activity was estimated by using standard method (Prieto *et al.,* 1999), Reducing Power assay was estimated was done according to the method described (Oyaizu 1986),1, 1diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity was estimated according to Wong *et al.,* (2006).

4.4 GC -MS analysis of fungal metabolites

Crude methanol extract of *F. globosum* was used isolate secondary metabolites by GC-MS analysis. Shimadzu scientific GC-MS-TQ8040NX, Japan was used for the analysis. The mass spectra of the compounds found in the extracts were matched with the National Institute of Standards and Technology (NIST) 17 libraries (Sathyamurthy *et al.,* 2014).

4.5 FT- IR Analysis of fungal metabolites

Methanol extract of isolated fungal metabolite was used for the ATR - FTIR analysis (Geesey, 1993).

Results and Discussion

Qualitative analysis of ethyl acetate extract of fungal metabolite showed the presence of alkaloids, phenols, flavonoids, steroids and tannins but terpenoid content was absent. Saponins and alkaloids were absent in methanolic extract. Ethyl acetate extract of *Alternaria alternata, Thielaviopsis basicola, Geotrichum albida, Penicillium frequentans* showed the presence of alkaloids, phenols, flavonoids, tannins and terpenoids but steroid was absent (Bhardwaj *et al.,* 2015). The active metabolites contain chemical groups such as phenols, steroids, flavonoids, quinines, terpenoids, xantones, peptides, cytocatalasins, alkaloids, aliphatic compounds, and phenyl propanoids (Alurappa and Chowdappa, 2018).

In quantitative assay, total phenol content was high (145.48±0.01 mg/g) in ethyl acetate extract and low (90.92 ±0.030 mg/g) in methanolic extract. The flavonoid content was found to be higher in methanolic extract (33.17± 0.03 mg/g) and lower (27.55±0.02 mg/g) in ethyl acetate extract. Methanolic extract showed high (153.36±2.32 mg/g) amount of tannin compared to ethyl acetate extract (32.11±0.01 mg/g). Fungi isolated from *Tabebuia argentea* different solvent extracts showed the presence of tannins, flavonoids, steroids, alkaloids, phenols and proteins)Dhankhar, 2012(. Ethyl acetate extracts of *C. gloeosporioides* showed the presence of alkaloids and steroids, whereas *F. oxysporum* extract revealed the presence of flavonoids, phenol and phenolic compounds (Ramesha and Srinivas, 2014). Alkaloids, steroids, tannins, flavonoids and phenolic content were present in the fungal metabolite of ethyl acetate extract of *Pinus roxburghii*)Wogu and Ofuase, 2014(. The fungus *Geotrichium albida* isolated from *Plumeria acuminata* and *Plumeria obtusifolia* showed the presence of alkaloids, flavonoids, steroids and phenolic compounds (Kamarian and Ghasemlou, 2013).

Total antioxidant activity was found high (33.16± 0.045 mg/g) in methanolic extract of metabolite of *Fusarium globosum***.** Reducing power was found to be higher (8.17± 0.035 mg/g) in methanolic extract of fungal metabolite. Methanolic extract of fungal metabolite showed higher (0.37±0.003 mg/g) DPPH activity compared to ethyl acetate extract (0.31±0.01 mg/g). The antioxidant activity of *N. sphaerica* isolated from *Artemisiaherba alba* and *Chiliadenus montanus* was 14% and 13%, respectively at the conc. of 1000 µg/mL (Selim *et al.,* 2018). The ethyl acetate extract of *S. commune* exhibited higher (15 ± 0.88 µg/ml) antioxidant activity (Prima *et al.,* 2022). Namvar *et al*., (2017) was reported that the ratio of methanol/water (80:20 v/v) was effective solvent in the extracting of phenolic compounds. *S. turcomanica* extracts have shown a good correlation

between antioxidant activity and total phenolic content. To release of many secondary metabolites, methanol was the best solvent (Namvar *et al.*, 2017). When compared to other solvent extracts, the methanol extract was found to exhibit the highest scavenging activity. Methanol extract possesses higher antioxidant molecules such as phenolic compounds. When compared to an intermediate polar solvent, acetone, and nonpolar solvent, hexane, methanol was very effective in extracting more antioxidant compounds (Madla *et al.*, 2012).

The secondary metabolites were determined as area percentage value with different peaks and retention time by GC-MS. Total 30 bioactive compounds with their corresponding peaks have been isolated from the metabolite of *F. globosum*. Ergosterol)14.25(, n-Hexadecanoic acid)13.32(and oleic Acid)11.30(were found to be higher followed by 2-)Dimethylamino(ethyl vaccenoate)0.30(in methanolic extract of *F. globosum.* In the past few years, Gas chromatography-Mass spectrometry (GC-MS) is used as one of the technological platforms for fingerprint analysis of secondary metabolites in both plant and non-plant species (Spiteller, 2015). GC-MS identification showed that fraction four of fungal extract has 19 detectable compounds from *A. coenophialum* (Hateet, 2020). Eleven compounds were detected from the ethyl acetate crude extract of *Curvularia aeria* isolated from the *Tribulus terrestris* and the result revealed the presence of Palmitic acid (24.54%), Hexadecanoic acid, Z-11-(10.57%), E,E-6,8-Tridecadien-2-ol, acetate (8.71%), Tetradecanoic acid, 12-methyl-, methyl ester (8.50%), 1-Eicosene (7.93%), Spirostan-9-ol, 3-amino-, (3a,5a25R)-(6.95%), 9-Amino-6-fluoro-lepidine (7.14%), Z-13-Octadecen-1-yl acetate (6.79%), c-Thionodecalactone (6.76%), 9-oxabicyclo (3.3.1) nonan-2-one, 6-hydroxy-(6.29%), Dimethyl 1-phenyl-7-methyl-1-hydroxy-1,4-dihydronaphthalene-2,3-dicarboxylate (5.76%) (Sahani and Thakur, 2019).

The presence of 19 different functional groups have been reported from ATR - FTIR analysis. FT-IR spectroscopy is a non-destructive technique allows to examine the total biochemical profile of intracellular metabolites in fungal cells as well as extracellular metabolites by using high-throughput screening FT-IR measurements)Gorain, 2018(. In amide I and amide II regions, considerable variation of *Phoma herbarum* and *T. piluliferum* was observed. *H. lixii* and *M. guilliermondii* were able to show high variation in N-H stretching of proteins (Dzurendova, 2020(. FTIR spectroscopy delivers a combined advantage for efficient classification of fungi as well as simultaneous visualization of chemical composition of the sample)Salman, 2010(.

Chapter 5: Evaluation of cell wall degrading enzyme activity of fungal isolates

Materials and Methods

5.1 Qualitative analysis of cell wall degrading enzymes

Fungal isolates were used to check the activity of cellulase, amylase and pectinase (Maria *et al.*, 2005: Yoon *et al.*, 2007: Beg *et al.*, 2000).

5.2 Quantitative analysis of Amylases, Cellulases and Pectinase

Amylase Activity was analyzed using standard method (Kathiresan and Manivannan, 2006), Cellulase activity was done according to the method described (Ghoose, 1987), Pectinase activity was analyzed according to Miller, 1959.

5.3 Optimization of amylase, cellulase and pectinase activity.

The optimization of amylase, cellulase and pectinase activity was measured at 10℃, 30℃ and 50℃ (Miller, 1959; Ghoose, 1987; Kathiresan and Manivannan, 2006).

5.4 LC - MS analysis

Methanolic extracts of fungal isolates were used for LC-MS analysis. LC-MS analysis was done using HPLC: Shimadzu C18 column. The mobile phase was acetonitrile and water containing 0.1% formic acid. The flow rate was 0.5 mL/min and the column temperature was maintained at 40℃. The peaks were detected by the detector UV 254 nm (John *et al.*, 2002).

Results and Discussion

Colour zone around all the fungal colonies showed the presence of starch thus indicating the activity of amylase. Presence of pale yellow hallow zone around the colony indicated the cellulase activity. Cellulase was absent in *Aspergillus niger*. Pectinase activity was present showing the colour zones around the colonies and no pectinase activity was observed in *Geotrichum candidum*. In the field of biomass utilization, cellulase enzyme provide a huge opportunity (Wen, 2005). Industrially relevant enzymes have enhanced interest in the exploration of extracellular enzymatic activity by the potential of using microorganisms as biotechnological sources (Abu, 2005). Both acidic and alkaline conditions of the organism produced significant level of inducible amylase enzyme and the cellulase was lower compared with other enzymes (Niturea *et al.*, 2008). Various plant pathogenic fungi such as *F.oxysporum, Botrytis cinerea, Sclerotinia sclerotiorum* are capable of secretion of pectin degrading enzymes during infection to the plants (Tenhave *et al.*, 2001; Heras *et al.*, 2003; Li *et al.*, 2004). Starch degrading properties of *Aspergillus flavus, Rhizopus species, Mucor* species, *Mucor mucedo, Aspergillus niger, Curvularia, Pencillum, Syncephalastrum*

racemosum, Rhizomucor and *Fusarium* species have been reported. *A. flavus, Mucor species, Mucor mucedo* and *Rhizopus* species have shown cellulase degrading properties but high amylase degradative property was recorded from *Curvularia*. Lack of cellulose degrading property was observed in *Fusarium* sp, *Aspergillus niger, Curvularia, Pencillum, Syncephalastrum racemosum* and *Rhizomucor* (Kavya *et al.,* 2019).

Amylase was found high in *Mucor irregularis* (116. 7 ±1.101 µg/mL/min) followed by *Pichia kudriavzeii* (112.9±1.5 µg/mL/min), *Aspergillus flavus* (90.95±1.09 µg/mL/min), *Geotrichum candidum* (74±1 µg/mL/min), *Aspergillus niger* (58.30±0.090 µg/mL/min), *Fusarium globosum* (56.33±1.12 µg/mL/min), *Aspergillus fumigatus* (33.62 ± 0.040 µg/mL/min), *Ganoderma lucidum* (24. 26 ±0.37 µg/mL/min). Cellulase was found high in *Geotrichum candidum* (312. 06 ± 0.901 µg/mL/min) followed by *Pichia kudriavzeii* (109.4±0.40 µg/mL/ min), *Fusarium globosum* (104. 33 ±1.52 µg/mL/min), *Aspergillus flavus* (52.27±0.15 µg/mL/ min), *Mucor irregularis* (43.68±0.03 µg/mL/min), *Ganoderma lucidum* (33. 1 ±1 µg/mL/min), *Aspergillus fumigatus* (26.47±1.17 µg/mL/min). Pectinase was recorded high in *Aspergillus flavus* (92.33 ±0.251 µg/mL/min) followed by *Fusarium globosum* (71.23±0.01 µg/mL/min), *Pichia kudriavzeii* (65.8±1.05 µg/mL/min), *Aspergillus fumigatus*(61.19±0.940 µg/mL/min), *Mucor irregularis* (54.74±2.05 µg/mL/min), *Aspergillus niger* (54.02±3.24 µg/mL/min), *Aspergillus niger* (54.02±3.24 µg/mL/min). Majority of fungal isolates *viz., Aspergillus, Fusarium, Alternaria, Rhizopus, Penicillum* and *Trichoderma* were found to possess cellulolytic activity. Wide range of enzymes produced by *Aspergillus* capable of degrading plant cell wall polysaccharide. A wide range of *Aspergillus* species have been identified to possess all component of cellulase enzyme system (Vries and Visser, 2001). *Trichoderma ressei, T. koningii, Fusarium* sp., *Aspergillus* and *Penicillium* sp., are the most common and most potent cellulase producers (Yalpani, 1987). The most common fungi isolated were *Aspergillus* sp., *Rhizopus* sp., *Mucor* sp., and *Fusarium* were isolated from mango fruits as post-harvest fungi (Gadgile *et al.*,2011). The growth and enzymatic activities of microorganisms are greatly affected by the temperature and the pH of the extracellular environment (Hyde *et al.,* 2019). *A. niger* and *A. flavus* were able to produce the cellulase in moderate to poor respectively (Asrat e*t al.,* 2018). The reduction of cellulase production and activity of CWDE of pathogens is directly related to the reduction of their invasiveness and pathogenicity (Khaledi and Hassani, 2018).

Optimization of amylase, cellulase and pectinase activity was measured at 10℃, 30℃ and 50℃. The amylase activity of *Fusarium globosum* was more at 30℃ (61.33±3.05 µg/mL/min) followed by 50℃ (46 ± 1 µg/mL/min), 10℃ (32±2 µg/mL/min). *Aspergillus fumigatus* was more at 30℃ (42.66±1.154 µg/mL/min), 50℃ (41.66±1.52 µg/mL/min), 10℃ (32.66±1.52 µg/mL/min). *Aspergillus flavus* 30℃ (95.66±2.08 µg/mL/min), 50℃ (63.66±1.52 µg/mL/min), 10℃ (61.66±1.52 µg/mL/min). *Mucor irragularis* was more at 30℃ (126±1 µg/mL/min), 10℃ (68.33±1.52 µg/mL/min), 50℃ (45.66±2.08 µg/mL/min). *Aspergillus niger* was more in 50℃ (90±2 µg/mL/min), 10℃(70.33±0.57 µg/mL/min), 30℃ (65.33±1.154 µg/mL/min). *Ganoderma lucidum* was more in 50℃ (37.66±1.52 µg/mL/min) , 10℃ (32.66±1.15 µg/mL/min), 30℃ (32±1 µg/mL/min). *Pichia kudriavzei* was more in 50℃ (182.3 ± 2.08 µg/mL/min), 10℃(111 ± 1 µg/mL/min) 30℃ (103.3±1.52 µg/mL/min). *Geotrichum candidum* was more at 30℃ (76 ± 1 µg/mL/min), 50℃ (65.66±2.08 µg/mL/min), 10℃ (49 ± 1 µg/mL/min).

The cellulase activity of *Fusarium globosum* was more at 30℃ (123 ± 1 µg/mL/min) followed by 50℃ (43.33 ± 0.57 µg/mL/min), 10℃ (39.33±1.52 µg/mL/min). *Aspergillus fumigatus* was more at 50℃ (47.66±0.57 µg/mL/min), 30℃ (33.66±1.52 µg/mL/min), 10℃ (28.33±1.52 µg/mL/min). *Aspergillus flavus* 50℃ (82±2 µg/mL/min), 30℃ (54.33±0.57 µg/mL/min), 10℃ (35.33±1.15 µg/mL/min). *Mucor irragularis* was more at 50℃ (65.66±1. 52 µg/mL/min), 30℃(49.66±1.52 µg/mL/min), 10℃(49.33±1.154 µg/mL/min). *Ganoderma lucidum* was more in 50℃ (53.66±0.57 µg/mL/min) , 30℃(33±1 µg/mL/min), 10℃ (32. 33 ±0.57 µg/mL/min). *Pichia kudriavzei* was more in 30℃ (107.66 ± 0.57 µg/mL/min), 50℃(83.33 ± 0.57 µg/mL/min), 10℃ (68±1 µg/mL/min). *Geotrichum candidum* was more at 30℃ (320 ± 1 µg/mL/min), 50℃(241.33 ± 1.52 µg/mL/min), 10℃(221 ± 1 µg/mL/min).

The pectinase activity of *Fusarium globosum* was more at 30℃ (73.33±1.52 µg/mL/min) followed by 50℃ (60.36 ± 0.321 µg/mL/min), 10℃ (53.66 ± 1.52 µg/mL/min). *Aspergillus fumigatus* was more at 50℃ (64.5±0.40 µg/mL/min), 30℃ (62.83±1.60 µg/mL/min), 10℃(62±1 µg/mL/min). *Aspergillus flavus* was more in 10℃ (123.66±1.52 µg/mL/min), 50℃ (118.4 ± 0.404 µg/mL/min), 30℃ (91±1 µg/mL/min). *Mucor irragularis* was more at 10℃ (72±1 µg/mL/min), 50℃(66.23±2.08 µg/mL/min), 30℃ (51.66±2.08 µg/mL/min). *Aspergillus niger* was more in 10℃ (73.66±1.04 µg/mL/min), 50℃(68.4±0.360 µg/mL/min),

30℃ (50.66±3.05 µg/mL/min). *Ganoderma lucidum* was more in 50℃ (50±1 µg/mL/min), 30℃(49.5 ± 1.32 µg/mL/min), 10℃ (43.33±1. 52 µg/mL/min). *Pichia kudriavzei* was more in 10℃ (86.5 ± 0.493 µg/mL/min), 30℃ (67.9 ± 0.95 µg/mL/min), 50℃ (50.53±0.351 µg/mL/ min). Temperature is a significant controlling factor for enzyme production therefore temperature must be controlled as another critical parameter to get optimum enzyme activity **(**Akintobi *et al.*, 2012**)**. Enzyme activity is usually optimized and highly stabilized at certain temperature and the enzyme becomes inactive, slow or entirely denatured under above or below temperature (Lehninger *et al.*, 1992). Variations in optimum temperature have shown by different species of *Aspergillus* ranging from 30- 400 ℃ for pectinase activity, and it was regarded as the best range (Khatri *et al.*, 2015). The production of cellulase enzyme depends on optimal conditions like temperature, pH, etc. (Sulyman *et al.*, 2020). Different strains of *Aspergillus niger* were isolated from the decayed fruits and vegetables and industrially important enzymes like amylase, pectinase, lipase, cellulase, catalase, and urease were screened. High yield of pectinase production was recorded in *A. niger* (Rajeshwari and Suma, 2023).

LC-MS analysis of amylase of *Mucor irragularis*, cellulase of *Geotrichum candidum*, pectinase of *Aspergillus flavus* were analyzed. MS spectrum of *Mucor irragularis* and *Geotrichum candidum* exhibited abundant mass in 205.05, 223.20 and 220.20 respectively. Whereas, MS spectrum of *Aspergillus flavus* exhibited abundant mass in 223.20. For the identification of compounds found in the fungal species, LC-MS approach has been effectively investigated. Using LC-MS method and positive mode ionization of the mass spectrometer, ethyl acetate extracts of *Ochotonids ferulica* and *Pithoascus persicus* were analyzed. The total ion chromatogram of *O. ferulica* extract displayed molecular ion $[M]^+$ and protonated molecule $[M+H]^+$ at m/z 274.86 and 275.76, respectively (Tazik *et al.*, 2020).

Chapter 6: Antifungal properties of botanicals and bioassay of chitosan against isolated fungi.

Materials and Methods

6.1 Collection of botanicals and processing

Piper longum, Aristolochia indica, Cissampelos pareira, Spilanthes acmella, Alpinia galanga were collected from Malabar region of Kerala. Samples were collected in a sterile polythene bag and brought to the laboratory for the further study. Leaf materials were washed thoroughly under running tap water to remove dirt and rinsed with distilled water and dried in hot air oven and

powdered. 1 gm of each dried powder sample was extracted using aqueous, methanol, chloroform and hexane by Soxhlet extractor. Extracted samples were used for the further study.

6.2 Qualitative and quantitative phytochemical analysis of selected medicinal plants

Qualitative phytochemical analysis was carried out as per the standard method (Harborne, 1973).

6.3 Quantitative phytochemical analysis of selected medicinal plants

Total phenolic content was estimated according to Folin-Ciocalteu's phenol reagent method (Adedapo *et al.,* 2009; Nabavi *et al.,* 2008), Total flavonoid was estimated using Colorimetric aluminium chloride method (Ebrahimzadeh *et al.,* 2001; Nabavi *et al.,* 2008), Saponin content was estimated by following the method (Kim *et al.,* 2003), Alkaloids content was estimated by the method of Obadoni and Ochuko (2001), Tannin content was estimated according to the method of Price and Butler (1977).

6.4 Antioxidant activities of selected medicinal plants

Total antioxidant activity was carried out according to the following method (Prieto *et al.,* 1999).

Reducing Power assay was done according to the method described (Oyaizu 1986).

1, 1diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity was estimated by the method of Wong *et al.,* (2006). Free radical scavenging activity was estimated by ABTS method (Roberta *et al.,* 1999).

6.5 Effect of selected medicinal plants extracts on colony growth of isolated fungi by agar dilution technique

Effect of selected medicinal plants extracts on colony growth of isolated fungi by agar dilution technique (Naz *et al.,* 2012).

6.6 Minimum Inhibitory concentration (MIC) of selected medicinal plants against fungal isolates

Minimum inhibitory concentration (MIC) was measured according to Lachumy *et al.,* (2010). MIC is usually considered as the most laboratory measurement of the activity of antimicrobial agent against microorganisms. MIC was considered as the lowest concentration able to inhibit any fungal growth.

6.7 Chitosan activity on colony growth of isolated fungi

The activity of chitosan on colony growth of the isolated fungi was done according to Bhattacharya (2013) with slight modification.

Results and Discussion

Among aqueous, hexane, chloroform and methanol, the methanol was yielded good extract than the other solvents. The percentage yield of extract is *Piper longum*-19.33%, *Aristolochia indica*-21.42%, *Cissampelos pareira*- 29.41%, *Spilanthes acmella*- 23.10%, *Alpinia galanga*-24.31%.

Qualitative phytochemical analysis of selected medicinal plant extracts (Aqueous, Methanol, Chloroform, Hexane) revealed the presence carbohydrate, proteins, amino acids, cardiac glycosides, flavonoid, phenols, tannin, alkaloids, saponins, terpenoids, steroids. In quantitative assay, The total phenol content was high in *Spilanthes acmella* (104.25±0.069 mg/g) followed by *Piper longum* (102.71±0.26 mg/g), *Alpinia galanga* (91.40±0.27 mg/g), *Cissampelos pareira* (69.36±0.22 mg/g) and *Aristolochia indica* (69.72±0.24 mg/g). *Alpinia galanga* showed high amount of tannin (119.46±0.39 mg/g) followed by *Spilanthes acmella* (94.38±0.45 mg/g), *Piper longum* (75.01±0.020 mg/g), *Aristolochia indica* (65.4±0.41 mg/g) and *Cissampelos pareira* (61.27 ±0.22 mg/g). Flavonoid was found to be higher in *Cissampelos pareira* (0.69±0.03 mg/g) followed by *Piper longum* (0.64±0.005 mg/g), *Alpinia galanga* (0.58± 0.005 mg/g), *Aristolochia indica* (0.54±0.02 mg/g) and *Spilanthes acmella* (0.43±0.005 mg/g). High amount of alkaloid was recorded from *Cissampelos pareira* (0.97±0.006 mg/g) followed by *Aristolochia indica* (0.81±0.005 mg/g), *Spilanthes acmella* (0.65±0.02 mg/g), *Piper longum* (0.56±0.02 mg/g) and *Alpinia galanga* (0.47±0.02 mg/g). Saponin content was high in *Cissampelos pareira* (0.63±0.005 mg/g) followed by *Spilanthes acmella* (0.58± 0.005 mg/g), *Aristolochia indica* (0.47±0.02 mg/g), *Alpinia galanga* (0.43±0.005 mg/g) and *Piper longum* (0.41±0.005 mg/g).

Plant extract have been used worldwide for control of plant disease and extracts from many plant species were found to be involved in many pathogenic fungi control (Apisariyakul *et al.*, 1995; Zabka *et al.*, 2009; Prakash *et al.*, 2012; Askarne *et al.*, 2013; Ozbek *et al.*, 2020; 2021). Phytoconstituents like flavanoids, tannins, saponins and polyphenols are effective antimicrobial substances against a wide range of microorganisms. Some organic compounds of many medicinal plants contain bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids which provide definite physiological action on the human body (Edeoga *et al.*,2005). Many researchers are interested in medicinal plants for evaluation of antioxidant phytochemicals such as phenols, flavonoids and tannins which have received more attention for their potential role in prevention of human diseases (Upadhyay *et al.*, 2010). Different extracts of *H.*

madagascariensis showed the presence of glycosides, phenols, alkaloids, flavonoids, tannins, saponins, except aqueous extracts which lack cardiac glycoside and glycosides. Phenols, triterpenes and sterols were absent in aqueous extract of *C. barteri* (Biapa *et al.*, 2007). Phytochemicals constituents such as saponins, tannins, alkaloids, terpenoids, anthraquinones, phenolic compounds, cardiac glycosides, and flavonoids were reported from *Rumex abyssinicus*, *Cucumis pustulatus*, *Discopodium penninervium*, *Lippia adoensis*, *Euphorbia depauperata*, *Cirsium englerianum*, and *Polysphaeria aethiopica* (Kebede *et al.*, 2021).

The total antioxidant activity was found high in *Piper longum* (36.61±0.25 mg/g) followed by *Cissampelos pareira* (34.49±0.38 mg/g), *Aristolochia indica* (31.65±0.40 mg/g), *Alpinia galanga* (29.51±0.07 mg/g) and *Spilanthes acmella* (21.79±0.45 mg/g). *Aristolochia indica* showed high reducing power (45.88±0.105 mg/g) compared to *Cissampelos pareira* (45.0±0.105 mg/g), *Piper longum* (44.77±0.181 mg/g), *Alpinia galanga* (38.44±0.2 mg/g) and *Spilanthes acmella* (35.47±0.70 mg/g mg/g). The DPPH activity was found to be higher in *Alpinia galanga* (65.8±0.16 mg/g) followed by *Spilanthes acmella* (45.46±0.30 mg/g), *Piper longum* (44.29±0.22 mg/g), *Cissampelos pareira* (39.5±0.06 mg/g) and *Aristolochia indica* (35.47±0.70 mg/g). *Aristolochia indica* showed higher ABTS (56.37±0.23 mg/g) followed by *Piper longum* (53.40±0.43 mg/g), *Cissampelos pareira* (46.18±0.13 mg/g), *Alpinia galanga* (45.73±0.35 mg/g), *Spilanthes acmella* (33.49±0.37 mg/g).

Secondary metabolites of plants have been tested for their antimicrobial activity and a significant number of plant extracts and essential oils have been shown to possess antimicrobial activity (Rahman *et al.,* 2011). Flavonoids are found mainly in plants and may play an important role in controlling fungi. Isolated flavonoids from citrus species were found to have an inhibitory effect on fungal food contaminants *A. parasiticus, A. flavus, F. semitectum and P. expansum* (Salas *et al.,* 2011). Bioactive compounds that are present in different extracts encourages the studies on antioxidant, anti-inflammatory and antimicrobial (Tsuchiya *et al*, 1996, Ya *et al.,* 1998, Nabila *et al*., 2008). Different medicinal plants that are used traditionally in Iran have shown antioxidant activity against linoleic peroxidation and free radical scavenging activity on DPPH. *Biebresteinia multifida* has shown the most active antioxidant property against linoleic peroxidation with an IC 50 value of 1.28 mg/mL (Souri *et al.,* 2022).

A good radical scavenging activity was reported in *Mentha spicata* and *Curcuma zedoaria* (Alam *et al.,* 2006). Interest in utilization of natural antioxidants has been increased to reduce the

toxic effects of free radicals in the human body (Dinda *et al.*,2007). Medicinal plants are considered as natural antioxidants and are utilized in several medical applications because of the assurance of effectiveness and safety, free radical scavenging properties (Al-Snafi, 2016). Medicinal plants have long been used as safe, effective, and sustainable sources of natural antioxidants or free radical scavengers due to the presence of phenolic compounds such as phenolic acids, flavonoids, tannins, stilbenes, and anthocyanins compounds. Many pathological conditions such as cancer, diabetes, aging, cardiovascular, and other degenerative diseases, antioxidant activity of medicinal and food plants, making a marked contribution in the fight against these diseases (Uritu *et al.*, 2018).

Five different medicinal plant extracts were significantly effective in reducing the growth of fungi as compared with control plates. *A. niger* was resistant to Dichloromethane (DCM), aqueous and methanolic extracts of 14 plants (traditionally used in Paraguay) (Portillo *et al.*, 2001). Strong antifungal activity of *Bucida buceras, Breonadia salicina,* and *Xylotheca kraussiana* have been reported against *A. fumigatus* (Mahlo *et al.*, 2013). Ethyl acetate extracts of *Adhatoda vasica, Ocimum sanctum* and *Holoptelea integrifolia* have shown maximum antifungal activity against *Alternaria* sp., *Aspergillus parasi, Aspergillus nidulans, Trichoderma harzianum* and *Aspergillus flavus* (Ishnava *et al.*, 2012). Growth of 11 microorganisms such as *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Bordetella bronchiseptica, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus faecalis, Candida albicans, Aspergillus niger* and *Saccharomyces cerevisiae* inhibited by *Aristolochia indica* (Kumar *et al.*, 2006).

Methanol extracts of *Cissampelos pareira* and *Alpinia galanga* have shown minimum inhibitory concentration (MIC) against *Fusarium globosum* and *Pichia kudriavzeii* at the conc. of 5 µg/mL. Acetone and methanol extracts of *H. caffrum* showed good antifungal activity against the three most sensitive test organisms (with MIC values ranging between 0.02 and 0.08 mg/ml). Lower antifungal activity was observed in the DCM extract of *H. caffrum* against *T. harzianum* (MIC = 0.63 mg/ml). Four extracts (Hexane, dichloromethane, acetone and methanol) of *O. ventosa* had highest activity against *T. harzianum* (MIC values of 0.04 and 0.08 mg/ml). The acetone and DCM extracts of *V. infausta* also had moderate activity against *A. parasiticus* and *T. harzianum* (MIC values of 0.16 mg/ml) (Mahlo *et al.*, 2016). Out of the seven plant pathogens, only three fungi (*P. janthinellum, T. harzianum* and *F. oxysporum*) had significant sensitivity to the plant extracts (average MIC values of 0.28 mg/ml). *F. oxysporum* was reasonably sensitive. This fungus has been

reported to cause wilting diseases in several crops. *F. oxysporum* also causes the wilting disease of lentil, tomato and banana (Abd-Ellatif *et al.,* 2011). *Bucida buceras, Breonadia salicina,* and *Xylotheca kraussiana* have been reported to have strong antifungal activity against *A. fumigatus* (Mahlo *et al.,* 2013).

Chitosan showed the high reduction activity against the isolated fungi at the conc. 10 mg/L among different concentrations (10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L). Chitosan is a versatile compound with antimicrobial activity. Mode of action of chitosan have been investigated by several studies. As a natural fungicide, it affects germination and hyphal morphology of economically important post-harvest fungal pathogens (e.g., *Rhizopus stolonifer* and *Botrytis cinerea*). This polymer also inhibits the Growth of many other plant pathogenic and mycoparasitic fungi viz., *Alternaria* sp., *Colletotrichium* sp., or *Trichoderma* sp. are inhibited by the chitosan (Lopez-Moya, 2019). The complete inhibition of growth of *Alternaria consortialis, Clonostachys byssicola, Neofusicoccum parvum, Trichoderma harzianum,* and *Trichoderma asperellum* were observed on PDA plates amended with 50 mg/L of chitosan (Al-Najada and Gherbawy, 2015). Chitosan was markedly inhibited or completely prevented the growth of many other plant pathogenic or mycoparasitic fungi viz., *Rhizoctonia stolonifer, B. cinerea, Penicillium expansum,* and *Alternaria alternat*a (Junior *et al.,* 2012).

Chapter 7: Summary and Conclusion

In the present study, different fungal pathogens have been isolated from infected vegetables collected from the Malabar region of Kerala. A total of eight fungi *viz. Pichia kudriavzeii, Geotrichum candidum* (*Cucumis sativus*), *Fusarium globosum, Aspergillus fumigatus* (*Coccinia grandis*), *Ganoderma lucidum, Aspergillus niger* (*Momordica charantia*) and *Aspergillus flavus, Mucor irragularis* (*Trichosanthes cucumerina*) have been isolated from different vegetables. To confirm the fungal isolates responsible for the infection of the vegetables, ITS sequencing methods have been followed. Isolated fungi have shown the presence of cell wall degrading enzyme (cellulase, pectinase and amylase enzymes) activity by qualitative and quantitative assay. Among eight fungi, *F. globosum* was able to produce a metabolite on the culture plate. Hence, total thirty bioactive compounds have been screened through GC-MS and FT-IR analysis. Medicinal plants selected for the study have shown strong antifungal activity against fungal isolates. MIC conc. of selected plants have shown the strong inhibitory activity at the conc. of 5 µg/mL against fungal

isolates. This indicates that selected medicinal plants have the capacity to inhibit/ control the infections caused by various fungal pathogens due the presence of phytoconstituents.

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PUBLICATIONS

1. Anju Joseph and Chandra M (2022). Screening of secondary metabolites produced by *Fusarium globosum* isolated from infected *Coccinia grandis* of Malabar region of Kerala, India. *Mycopathological research*,60(3),417-425.

2. Anju Joseph and Chandra M (2022). Molecular characterization and enzymatic activity of *Pichia kudriavzevii* isolated from *Cucumis sativus* of Malabar region of Kerala. *Journal of Mycology and Plant Pathology*,52 (1): (ACCEPTED)

PRESENTATIONS

1. Anju Joseph and Chandra M (2020). “Isolation and identification of fungi from soil and infected vegetable; A review”. Alumini sponsored National conference on Current advance in Medical and Industrial Biotechnology organized by Department of Biotechnology Alva’s College Moodbidri in association with Karnataka state pollution control board during 20 February 2020.
2. Anju Joseph and Chandra M (2021). “Isolation and identification of fungi and analysis of secondary metabolites of *Fusarium globosum* from *Coccinia grandis* of Malabar regions of Kerala" during the virtual National Conference on Biodiversity and biotechnology of fungi and 47th annual meeting of mycological society of India February 22-24, 2021 at Department of Botany, Punjabi University, Patiala.
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